

## Crystal Structure of the *E. Coli* Thioesterase II, a Homology of the Human NEF Binding Enzyme

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The crystal structure of the *Escherichia coli* thioesterase II (TEII) has been solved using SeMet MAD technique at 1.9 Å resolution. *E. coli* TEII is a representative of a novel and ubiquitous family of thioesterases, members of which have been found in bacteria, yeast and humans. The amino acid sequence of this enzyme has been rather stringently conserved throughout evolution, such that the *E. coli* and human proteins are 45% identical, suggesting that it has an important physiological function. The human enzyme has been discovered in T cells, where it is one of the binding targets of the HIV-1 Nef protein, suggesting that it has a possible role in AIDS pathogenesis. The structure of the enzyme has a novel fold consisting of two repeats of an  $\alpha/\beta$  motif with a six-stranded antiparallel  $\beta$ -sheet topology. The motif is highly reminiscent of the tertiary structure of the 4-hydroxybenzoyl-CoA thioesterase, although no evidence of homology can be detected at the amino acid level, and the two enzymes apparently operate by different catalytic mechanisms. The active site of TEII shows novel chemistry for a thioesterase; it contains a hydrogen-bonding network of several residues, notably a triad of Asp 204, Gln 278 and Thr 228, which orient a water molecule for nucleophilic attack on the substrate. The 1.9 Å resolution structure of the *E. coli* enzyme sets the stage for further analysis of the structure-function relationships in this family of thioesterases, including the determination of the mechanism by which Nef binds to and activates the human enzyme.